

AMENDMENTS TO THE CLAIMS

1. (withdrawn): An isolated chimeric protein, which chimeric protein comprises, from N-terminus to C-terminus:

- a) a first peptidyl fragment comprising a bacterial leader sequence from about 5 to about 30 amino acid residues; and
- b) a second peptidyl fragment comprising a 3'(2'),5'-bisphosphate nucleotidase.

2. (withdrawn): The isolated chimeric protein of claim 1, wherein the bacterial leader sequence is a leader sequence of an *E. coli* protein.

3. (withdrawn): The isolated chimeric protein of claim 1, wherein the leader sequence has at least 40% identity to the amino acid sequence set forth in SEQ ID NO:1 (MGGSGDDDLAL), in which the percentage identity is determined over an amino acid sequence of identical size to the amino acid sequence set forth in SEQ ID NO:1.

4. (withdrawn): The isolated chimeric protein of claim 1, wherein the leader sequence binds to an antibody that specifically binds to an amino acid sequence set forth in SEQ ID NO:1.

5. (withdrawn): The isolated chimeric protein of claim 1, wherein the leader sequence comprises the amino acid sequence set forth in SEQ ID NO:1.

6. (withdrawn): The isolated chimeric protein of claim 1, wherein the first peptidyl fragment comprises about 20 amino acid residues.

7. (withdrawn): The isolated chimeric protein of claim 1, wherein the nucleotidase is 3'(2'),5'-bisphosphate nucleotidase.

8. (withdrawn): The isolated chimeric protein of claim 7, wherein the 3'(2'),5'-bisphosphate nucleotidase is of yeast, bacterial, or mammalian origin.

9. (withdrawn): The isolated chimeric protein of claim 1, wherein the nucleotidase has at least 40% identity to the amino acid sequence set forth in SEQ ID NO:2
(ALERELLVATQAVRKASLLTKRIQSEVISHKDSTTITKNDNSPVTG DYAAQTIIINAISNF PDDKVVGEESSSGLSDAFVSGILNEIKANDEVYNKNYKKDDFLFTNDQFPLKSLEDVRQIID FGNYEGGRKGRFWCLDPIDGPKGFLRGEQFAVCLALIVDGVVQLGCIGCPNLVLSSYGAQ DLKGHESFGYIFRAVRLGAFYSPSSDAESWTKIHVRHLKDTKDMITLEGVEKGHSSHDEQ TAIKNKLNISKSLHLD SQAKYCLLALGLADYVRLPIKLSYQEKIWDHAAGNVIVHEAGGI HTDAMEDVPLDFGNGRTLATKGVIASSGPRELHDLVVSTCDVIQSRNA), in which the percentage of identity is determined over an amino acid sequence of identical size to the amino acid sequence set forth in SEQ ID NO:2.

10. (withdrawn): The isolated chimeric protein of claim 1, wherein the nucleotidase binds to an antibody that specifically binds to an amino acid sequence set forth in SEQ ID NO:2.

11. (withdrawn): The isolated chimeric protein of claim 1, wherein the nucleotidase comprises the amino acid sequence set forth in SEQ ID NO:2.

12. (withdrawn): The isolated chimeric protein of claim 1, wherein the first and second peptidyl fragments are linked via a cleavable linkage.

13. (withdrawn): The isolated chimeric protein of claim 1, which further comprises, at its C-terminus, a third peptidyl fragment comprising a second bacterial leader sequence from about 5 to about 30 amino acid residues.

14. (withdrawn): The isolated chimeric protein of claim 13, wherein the second bacterial leader sequence is a leader sequence of an *E. coli* protein.

15. (withdrawn): The isolated chimeric protein of claim 13, wherein the second bacterial leader sequence has at least 40% identity to the amino acid sequence set forth in SEQ ID NO:3

(KGELEGLPIPPLLRTG), in which the percentage identity is determined over an amino acid sequence of identical size to the amino acid sequence set forth in SEQ ID NO:3.

16. (withdrawn): The isolated chimeric protein of claim 13, wherein the second bacterial leader sequence binds to an antibody that specifically binds to an amino acid sequence set forth in SEQ ID NO:3.

17. (withdrawn): The isolated chimeric protein of claim 13, wherein the second bacterial leader sequence comprises the amino acid sequence set forth in SEQ ID NO:3.

18. (withdrawn): The isolated chimeric protein of claim 13, wherein the third peptidyl fragment comprises about 20 amino acid residues.

19. (withdrawn): The isolated chimeric protein of claim 1, which further comprises at its C-terminus, a third peptidyl fragment comprising a peptide tag.

20. (withdrawn): The isolated chimeric protein of claim 19, wherein the peptide tag is selected from the group consisting of FLAG, HA HA1, c-Myc, 6-His, AU1, EE, T7, 4A6, ε, B, gE, and Ty1 tag.

21. (withdrawn): The isolated chimeric protein of claim 13, which further comprises, at its C-terminus a fourth peptidyl fragment comprising a peptide tag.

22. (withdrawn): The isolated chimeric protein of claim 21, wherein the peptide tag is selected from the group consisting of FLAG, HA HA1, c-Myc, 6-His, AU1, EE, T7, 4A6, ε, B, gE, and Ty1 tag.

23. (withdrawn): The isolated chimeric protein of claim 1, which comprises the amino acid sequence set forth in SEQ ID NO:4

(mggsgdddlalALERELLVATQAVRKASLLTKRIQSEVISHKDSTTITKNDNSPVTTG DYAAQT

IIINAICKSNFPDDKVVGEESSSGLSDAFVSGILNEIKANDEVYNKNYKKDDFLFTNDQFPLKS
LEDVRQIIDFGNYEGGRKGRFWCLDPIDGPKGFLRGEQFAVCLALIVDGVVQLGCIGCPNL
VLSSYGAQDLKGHESFGYIFRAVRGLGAFYSPSSDAESWTKIHRHLKDTKDMITLEGVEK
GHSSHDEQTAIKNKLNIKSLLHLDSSQAKYCLLALGLADVYLRLPKLSYQEKIWDHAAGNV
IVHEAGGIHTDAMEDVPLDFGNRGLATKGVIASSGPRELHDLVVSTSCDVIQSRNAkgeegl
pipnpllrtrghhhhhh).

24. (withdrawn): An isolated nucleic acid comprising a nucleotide sequence encoding the chimeric protein of claim 1.

25. (withdrawn): An isolated nucleic acid comprising a nucleotide sequence encoding the chimeric protein of claim 23.

26. (withdrawn): The nucleic acid of claim 24, which comprises the nucleotide sequence set forth in SEQ ID NO:5

(atggcgatccggatcggtatgacgtacccctGCATTGGAAAGAGAATTATTGGTGCACACTCAAGC
TGTACGAAAGGCGTCTTATTGACTAAGAGAATTCAATCTGAAGTGATTCTCACAGG
ACTCCACTACTATTACCAAGAATGATAATTCTCCAGTAACCACAGGTGATTATGCTGCA
CAAACGATCATCATAAATGCTATCAAGAGCAATTTCCTGATGATAAGGTAGTTGGTGA
AGAACCTCATCAGGATTGAGCGACGCATTCTCAGGAATTAAACGAAATAAAA
GCCAATGACGAAGTTATAACAAGAATTATAAAAAGGATGATTTCTGTTACAAACG
ATCAGTTCCGCTAAAATCTTGGAGGACGTCAGGCAAATCATCGATTCGGCAATTAC
GAAGGTGGTAGAAAAGGAAGATTTGGTGTGATCCTATTGACGGAACCAAGGGT
TTTAAGAGGTGAACAGTTGCAGTATGTCAGGCTTAATTGTCAGGACGGTGTGTCAG
CTTGGTTGTATTGGATGCCCAACTTAGTTAAGTTCTATGGGGCCCAAGATTGAA
AGGCCATGAGTCATTGGTTATCTTCGTGCTGTTAGAGGTTAGGTGCCTCTATT
TCCATCTCAGATGCAGAGTCATGGACCAAAATCCACGTTAGACACTTAAAGACACT
AAAGACATGATTACTTAGAGGGAGTTGAAAAGGGACACTCCTCTCATGATGAACAAA
CTGCTATCAAAAACAAACTAAATATCCAAATCTTGCACGGATTCTCAAGCCAAG
TACTGTTGTTAGCATTGGGCTTAGCAGACGTATTTACGTCTGCCTATCAAACCTTCT

TACCAAGAAAAGATCTGGGACCATGCTGCAGGCAACGTTATTGTCCATGAAGCTGGAG
GTATCCATACAGATGCCATGGAAGATGTTCTCTAGACTTCGTAACGGTAGAACGCTA
GCTACGAAGGGAGTTATAGCGTCAAGTGGCCCACGCGAGTTACATGACTTGGTGGTGT
CTACATCATGCGATGTCATTCAAGAACGCCaaggcgagcttgaaggttgcctatccctaaccctctc
ctccgtaccggcatcatcaccatcaccattga).

27. (withdrawn): An isolated nucleic acid comprising a nucleotide sequence complementary to the nucleotide sequence of claim 24.

28. (withdrawn): A recombinant cell containing the nucleic acid of claim 24.

29. (withdrawn): A method of producing a chimeric protein comprising growing a recombinant cell containing the nucleic acid of claim 24 such that the encoded chimeric protein is expressed by the cell, and recovering the expressed chimeric protein.

30. (withdrawn): The product of the method of claim 29.

31. (currently amended): A method for assaying for sodium ions in a sample, which method comprises:

a) contacting the sample with a sodium-sensitive 3'(2'),5'-bisphosphate nucleotidase, wherein the nucleotidase consumes adenosine 3',5'-bisphosphate (PAP) and forms AMP and P_i; and
b) assessing the consumption of PAP or the formation of AMP and P_i or P_i in step a) to determine the presence or amount of sodium ions in the sample.

32. (original): The method of claim 31, wherein the sample is a biological sample.

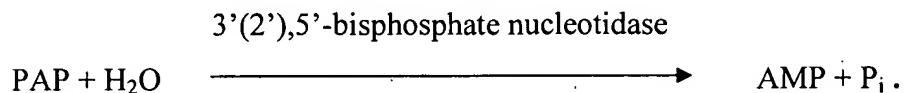
33. (original): The method of claim 32, wherein the biological sample is a blood sample.

34. (original): The method of claim 33, wherein the blood sample is a plasma, serum, red blood cell, or whole blood sample.

35. (previously presented): The method of claim 31, wherein the 3'(2'),5'-bisphosphate nucleotidase is an isolated chimeric protein, which chimeric protein comprises, from N-terminus to C-terminus:

- a) a first peptidyl fragment comprising a bacterial leader sequence from about 5 to about 30 amino acid residues; and
- b) a second peptidyl fragment comprising a 3'(2'),5'-bisphosphate nucleotidase.

36. (previously presented): The method of claim 31, wherein the 3'(2'),5'-bisphosphate nucleotidase is an enzyme that catalyzes the following reaction:



37. (original): The method of claim 31, wherein the amount of AMP formed is inversely related to the amount of sodium ions in the sample.

38. (original): The method of claim 31, which is used in prognosis or diagnosis of a disease or disorder.

39. (original): A method for assaying for sodium ions in a sample, which method comprises:

- a) contacting the sample with a first composition comprising adenosine 3',5'-bisphosphate (PAP);
- b) contacting the sample with a second composition comprising a sodium-sensitive 3'(2'),5'-bisphosphate nucleotidase; and
- c) assessing the production of AMP to determine the presence or amount of sodium ions in the sample.

40. (original): The method of claim 39, wherein the sample is a biological sample.

41. (original): The method of claim 40, wherein the biological sample is a blood sample.

42. (original): The method of claim 41, wherein the blood sample is a plasma, serum, red blood cell, or whole blood sample.

43. (previously presented): The method of claim 39, wherein the 3'(2'),5'-bisphosphate nucleotidase is an isolated chimeric protein, which chimeric protein comprises, from N-terminus to C-terminus:

- a) a first peptidyl fragment comprising a bacterial leader sequence from about 5 to about 30 amino acid residues; and
- b) a second peptidyl fragment comprising a 3'(2'),5'-bisphosphate nucleotidase.

44. (original): The method of claim 39, wherein the first composition further comprises 4-aminoantipyrine (4-AA), N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-m-toluidine (EHSPT), purine nucleoside phosphorylase, xanthine oxidase, and peroxidase, and the second composition further comprises adenosine deaminase, 5'-nucleotidase, and MgCl₂.

45. (previously presented): A kit for assaying for sodium ions in a sample, which kit comprises

- a) a first composition comprising a sodium-sensitive 3'(2'),5'-bisphosphate nucleotidase that consumes adenosine 3',5'-bisphosphate and forms AMP and P_i; and
- b) means for assessing the product formed or the substrate consumed by the nucleotidase to determine the presence or amount of the sodium ions in the sample.

46. (original): The kit of claim 45, wherein the first composition further comprises adenosine deaminase, 5'-nucleotidase and MgCl₂.

47. (previously presented): The kit of claim 45, further comprising a second composition comprising 4-aminoantipyrine (4-AA), N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-m-toluidine (EHSPT), purine nucleoside phosphorylase, xanthine oxidase, and peroxidase, wherein the reaction of 4-AA and EHSPT in the presence of peroxidase is the means for assessing the product formed.

48. (original): The kit of claim 45, which further comprises a low sodium serum standard and a high sodium serum standard.

49. (previously presented): The kit of claim 45, wherein the 3'(2'),5'-bisphosphate nucleotidase is an isolated chimeric protein, which chimeric protein comprises, from N-terminus to C-terminus:

- a) a first peptidyl fragment comprising a bacterial leader sequence from about 5 to about 30 amino acid residues; and
- b) a second peptidyl fragment comprising a 3'(2'),5'-bisphosphate nucleotidase.

50. (previously presented): A method for assaying for lithium ions in a sample, which method comprises:

- a) contacting the sample with a lithium-sensitive 3'(2'),5'-bisphosphate nucleotidase, wherein the nucleotidase consumes adenosine 3',5'-bisphosphate (PAP) and forms AMP and Pi; and
- b) assessing the amount of PAP consumed or AMP or Pi formed in step (a) to determine the presence or absence of lithium ions in the sample.

51. (original): The method of claim 50 further comprising first contacting the sample with a sodium blocking agent.

52. (original): The method of claim 51, wherein the sodium blocking agent is 4, 7, 13, 16, 21-pentaoxa-1,10-diazabicyclo[8.8.5]-tricosane.

53. (original): The method of claim 51, wherein the sample is a biological sample.

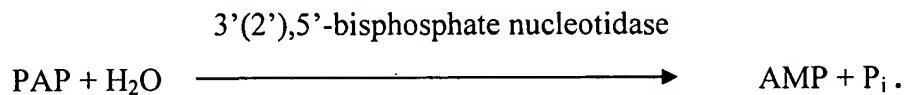
54. (original): The method of claim 53, wherein the biological sample is a blood sample.

55. (original): The method of claim 54, wherein the blood sample is a plasma, serum, red blood cell, or whole blood sample.

56. (previously presented): The method of claim 51, wherein the 3'(2'),5'-bisphosphate nucleotidase is an isolated chimeric protein, which chimeric protein comprises, from N-terminus to C-terminus:

- a) a first peptidyl fragment comprising a bacterial leader sequence from about 5 to about 30 amino acid residues; and
- b) a second peptidyl fragment comprising a 3'(2'),5'-bisphosphate nucleotidase.

57. (previously presented): The method of claim 51, wherein the nucleotidase is an enzyme that catalyzes the following reaction:



58. (original): The method of claim 51, wherein the amount of AMP formed is inversely correlated to the amount of lithium ions in the sample.

59. (original): The method of claim 51, which is used in prognosis or diagnosis of a disease or disorder.

60. (previously presented): A method for assaying for lithium ions in a sample, which method comprises:

- a) contacting the sample with a first composition comprising adenosine 3',5'-bisphosphate (PAP);
- b) contacting the sample with a second composition comprising a lithium-sensitive 3'(2'),5'-bisphosphate nucleotidase; and
- c) assessing the production of a detectable product to determine the presence or absence of lithium ions in the sample.

61. (original): The method of claim 60 further comprising first contacting the sample with a sodium blocking agent.

62. (original): The method of claim 61, wherein the sodium blocking agent is 4, 7, 13, 16, 21-pentaoxa-1,10-diazabicyclo[8.8.5]-tricosane.

63. (original): The method of claim 60, wherein the sample is a biological sample.

64. (original): The method of claim 63, wherein the biological sample is a blood sample.

65. (original): The method of claim 64, wherein the blood sample is a plasma, serum, red blood cell, or whole blood sample.

66. (previously presented): The method of claim 60, wherein the 3'(2'),5'-bisphosphate nucleotidase is an isolated chimeric protein, which chimeric protein comprises, from N-terminus to C-terminus:

- a) a first peptidyl fragment comprising a bacterial leader sequence from about 5 to about 30 amino acid residues; and
- b) a second peptidyl fragment comprising a 3'(2'),5'-bisphosphate nucleotidase.

67. (original): The method of claim 60, wherein the first composition further comprises 4-aminoantipyrine (4-AA), N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-m-toluidine (EHSPT), purine nucleoside phosphorylase, xanthine oxidase, and peroxidase, and the second composition further comprises adenosine deaminase, 5'-nucleotidase, and MgCl₂.

68. (previously presented): A kit for assaying for lithium ion in a sample, which kit comprises:

- a) a first composition comprising a lithium-sensitive 3'(2'),5'-bisphosphate nucleotidase; and
- b) a means for assessing the adenosine 3',5'-bisphosphate consumed or the AMP or Pi formed by the 3'(2'),5'-bisphosphate nucleotidase to determine the presence or amount of said lithium ions in the sample.

69. (previously presented): The kit of claim 68 further comprising a sodium blocking agent.

70. (original): The kit of claim 68, wherein the first composition further comprises adenosine deaminase, 5'-nucleotidase and MgCl₂.

71. (previously presented): The kit of claim 68, further comprising a second composition comprising 4-aminoantipyrine (4-AA), N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-m-toluidine (EHSPT), purine nucleoside phosphorylase, xanthine oxidase, and peroxidase, wherein the reaction of 4-AA and EHSPT in the presence of peroxidase is the means for assessing the product formed.

72. (original): The kit of claim 68, which further comprises a low lithium serum standard, a medium lithium sodium standard, and a high lithium serum standard.